Takashi MATSUSHIMA, Hiroshi ITŌ, and Mineko IKEDA*:

Investigation on the fungal spoilage of crude drugs (2)**

松島 崇:伊東 宏:池田峰子*:

絲状菌による生薬の変質に關する研究 (2)

Shakuyaku, PAEONIA (J.P.VI)***, root of *Paeonia albiflora* Pallas var. *trichocarpa* Bunge (Ranunclaceae) and its allied plants.

R. H. 75% : In 28 days, a trace of the fungal growth was observed.

Growing species: *Aspergillus mangini* (Mangin) Thom and Raper.

R. H. 84% : In 7 days, a slight development of *Aspergillus glaucus* group was observed. The growth in 28 days was relatively good.

Dominant species: *Aspergillus ruber*, *Aspergillus mangini* (Mangin) Thom and Raper.

R. H. 100% : A fungal growth was observed in 3 days. Until about 10 days, *Aspergillus glaucus* group was predominant, but later *Penicillium frequentans*, *Peniicllium implicatum* and subsequently *Penicillium variabile* developed. In 21 days, *Penicillium variabile* dominated over other fungi. The fungal development in 28 days was very good.

Dominant species: *Penicillium variabile* Sopp.

Subdominant species: *Aspergillus ruber*, *Aspergillus umbrosus* Bain and Sart. *Aspergillus mangini* (Mangin) Thom and Raper.

Minor species: *Penicillium frequentans* Westling, *Penicillium implicatum* Biourge.

Very minor species: *Aspergillus niger* van Tieghem. Unidentified fungus (yellow mycelia), *Rhizopus nigricans* Ehrenberg, *Aspergillus ochraceus* Wilhelm.

Kikyô, PLATYCODON (J.P. VI) root of *Platycodon grandiflorum* A. De Candole (Campanulaceae).

R. H. 75% : In 17 days, a fungal growth was observed. In 28 days, the fungal development was poor.

Growing species: *Aspergillus ruber*.

R. H. 84% : In 7 days, the growth of *Aspergillus glaucus* group became visible. The fungal development in 28 days was relatively good.

---

* Contribution from Division of Medical Plant Garden, National Hygenic Laboratory, Setagaya, Tokyo. 国立衞生試験所薬用植物部

** Part 1 was published in Journ. Jap. Bot. **32**: 201-207 (July 1957).

*** Japanese Pharmacopea Ed. VI.

Dominant species: *Aspergillus ruber.*

Minor species: *Aspergillus repens.*

R. H. 100%: The fungal growth was observed in 2 days. Until 7 days, *Aspergillus glaucus* group was predominant, later *Penicillium frequentans* and subsequently *Penicillium purprogenum* and *Penicillium duclauxi* developed rapidly. In 14 days, *Penicillium* spp. dominated over other fungi. The fungal growth in 28 days was very good.

Dominant species: *Penicillium purprogenum* Stoll, *Penicillium frequentans* Westling, *Penicillium duclauxi* Delacoiux.

Subdominant species: *Aspergillus ustus* (Bain.) Thom and Church.

Minor species: *Aspergillus ruber*, *Aspergillus repens*, *Aspergillus sulphureus* Fresenius, *Rhizopus nigricans* Ehrenberg, *Aspergillus candidus* Link, *Aspergillus niger* van Tieghem, *Trichothecium roseum* Link, *Aspergillus fumicola* Chad. and Sach.

Obaku, PHELLODENDRON (J.P.VI), cortex of *Phellodendron amurense* Ruprecht (Rutaceae) and its allied plants.

R. H. 75%: No fungal development in 28 days.

R. H. 94%: In 3 days, the development of *Aspergillus ruber* was observed. The growth in 28 days was very poor.

Growing species: *Aspergillus ruber.*

R. H. 100%: In 2 days, *Aspergillus repens* began to develop and predominated until about 10 days. Later *Aspergillus awamori* developed. The fungal growth in 28 days was fairly good.

Dominant species. *Aspergillus repens*, *Aspergillus awamori* Nakazawa.

Very minor species: *Aspergillus sclerotiorum* Huber, *Rhizopus nigricans* Ehrenberg, *Penicillium* sp. (Asymmetrica).

Kakkon, PUERARIA (J. P. VI) root of *Pueraria hirsuta* Matsumura (Leguminosae)

R. H. 75%: In 21 days, *Aspergillus restrictus* began to develop. The growth in 28 days was very poor.

Growing species: *Aspergillus restrictus* G. Smith

R. H. 84%: In 5 days, *Aspergillus glaucus* group began to develop. The growth in 28 days was very good.

Dominant species: *Aspergillus repens*, *Aspergillus ruber*, *Aspergillus restrictus* G. Smith.

Very minor species: white mycelia (*Monilia* sp. ?)

R. H. 100%: In 2 days, *Aspergillus ruber* began to develop. Retarding 2 days, *Aspergillus awamori* appeared. The growth in 28 days was very good.

Dominant species: *Aspergillus ruber*.

Subdominant species: *Aspergillus awamori* Nakazawa.

Very minor species: *Aspergillus ochraceus* Wilhelm.

Chôji, CARYOPHYLLUS (J. P. VI), flower bud of *Eugenia caryophylla* Thunberg (Myrtaceae).

R. H. 75%: No fungal development in 28 days.

R. H. 84%: No fungal development in 28 days.

R. H. 100%: No fungal development in 28 dgys.

Sanshô, ZANTHOXYLUM (J. P. VI), fruit of *Zanthoxylum piperitum* De Candolle (Rutaceae) and its allied plants.

R. H. 75%: No fungal development in 28 days.

R. H. 84%: In 7 days, *Aepergillus mangini* began to develop. The growth in 28 days was very poor.

Growing species: *Aspergillus mangini* (Mangin) Thom and Raper.

R. H. 100%: Until about 10 days, only *Aspergillus repens* was growing. In 14 days *Aspergillus versicolor* and subsequently *Penicillium implicatum* became significant and grew rapidly. The growth in 28 days was fairly good.

Dominant species: *Aspergillus versicolor* (Vuill.) Tiraboschi.

Subdominant species: *Penicillium implicatum* Biourge, *Aspergillus repens*.

Minor species: *Aspergillus ochraceus* Wilhelm, *Aspergillus miyakoensis* Nak., Simo. & Wat., *Trichothecium roseum* Link.

Very minor species: *Memnoniella echinata* (Rivolta) Galloway, *Fusarium* sp., *Chaetomium globosum* Kunze.

Shôkyô, ZINGIBER (J. P. VI), subterranean stem of *Zingiber officinale* (Zingiberaceae).

R. H. 75%: No fungal development in 28 days.

R. H. 84%: Until about 25 days, no fungal development was observed, but later *Fumicola* sp. developed very poorly.

Growing species: *Fumicola* sp.

R. H. 100%: In 5 days, *Penicillium frequentans* began to develop. Retarding 2 days, *Aspergillus flavus* developed. The fungal growth after 28 days was good.

Dominant species: *Penicillium frequentans* Westling.

Subdominant species: *Aspergillus flavus* Link.

Minor species: *Penicillium wortmanni* Klöcker.

Very minor species: *Aspergillus ruber*.

Tônin, PERSICA (N. F. J. II),[***] seed of *Prunus persica* Batsch (Rosaceae) and its allied plants.

R. H. 75%: No fungal development in 28 days.

R. H. 84%: In 10 days, *Aspergillus umbrosus* began to develop and its growth in 28 days was very poor.

Growing species: *Asergillus umbrosus* Bainier and Sartory.

R. H. 100%: In 4 days, *Rhizopus nigricans* began to develop. In 14 days, *Trichoderma viride* began to develop. The fungal growth in 28 days was very good.

Dominant species: *Rhizopus nigricans* Ehrenberg.

Subdominant species: *Trichoderma viride* Pers. ex Fr. (growing on the substrate and *Rhizopus nigricans*).

Minor species: *Aspergillus ochraceus* Wilhelm.

Very minor species: *Aspergillus tamarii* Kita, *Aspergillus ruber*.

Bukuryô, HOELEN (N. F. J. II), large sclerotium of *Poria* spp. in pine root.

R. H. 75%: No fungal development in 28 days.

R. H. 84%: No fungal development in 28 days.

R. H. 100%: In 6 days, a fungal growth was observed, but its growth was quite retarded. The fungal growth in 28 days was poor.

Dominant species: *Penicillium purpurogenum* Stoll.

Minor species: *Penicillium rugulosum* Thom, *Aspergillus luchuensis* Inui.

Kanokosô, VALERIANA (J. P. VI), subterranean stem and root of *Valeriana officinalis* Linné var. *latifolia* Miquel (Valerianaceae).

R. H. 75%: In about 20 days, the growth of *Torula sacchari* and *Aspergillus restrictus* began to develop. The growth in 28 days was very poor.

Growing species: *Torula sacchari* Corda, *Aspergillus restrictus* G. Smith.

R. H. 84%: In 3 days, *Torula sacchari* began to develope and predominated hereafter. In about 10 days, *Aspergillus glaucus* group began to appear. The fungal development in 28 days was poor.

Dominant species: *Torula sacchari* Corda.

[***] National Formulary of Japan Ed. II.

Suadominant species: *Aspergillus restrictus* G. Smith, *Aspergillus ruber*.

Minor species: *Fumicola grisea* Traaen.

Very minor species: *Scopulariopsis brevicaulis* var. *glabrum* Thom.

Okera, Atractylodes (J. P. VI.), subterranean stem of *Atractylodes japonica* Koidzumi (Compositae).

R. R. 75%: In 28 days, the fungal growth was very poor.

Growing species: *Aspergillus ruber*, *Aspergillus ruber* NHL 5078 (This fungus produced white, silky floccose colonies on this substrate).

R. H. 84%: In 10 days, only *Aspergillus ruber* NHL 5078 was growing very poorly and later *Aspergillus glaucuus* group began to develop. The growth in 28 days was poor.

Dominant species: *Aspergillus ruber* NHL 5078.

Minor species: *Aspergillus restrictus* G. Smith, *Aspergillus ruber*, *Apspergillus amstelodami*.

Very minor species: *Penicillium cyclopium* Westling.

R. H. 100%: In 4 days, *Aspergillus ruber* NHL 5078 began to develop and dominated hereafter. In about 10 to 14 days, *Aspergillus ustus* and *Aspergillus niger* began to develop. The fungal growth in 28 days was very good. The good growth of *Aspergillus ruber* NHL 5078 and *Aspergillus ustus* gave a conspicuous appearence.

Dominant species: *Aspergillus ruber* NHL 5078.

Subdominant species: *Aspegillus ustus* (Bainier) Thom and Church.

Minor species: *Aspergillus niger* van Tieghem, *Penicillium cyclopium* Westling.

Very minor species: *Aspergillus sulphureus* (Fres.) Thom and Church, *Aspergillus versicolor* (Yuill.) Tiraboschi, *Aspergillus oryzae* (Ahlb.) Cohn, *Aspergillus ruber*, *Aspergillus repens*, *Aspergillus amstelodami*.

Kyônin, ARMENIACA (J. P. VI.), seed of *Prunus armeniaca* Linné var. *anzu* Maximowicz (Rosaceae).

R. H. 75%: In 28 days, no fungal growth was observed.

R. H. 84%: In 7 days, *Aspergillus repens* began to develop and predominated until about 21 days. Later *Penicillium viridicatum* began to develop. The fungal growth in 28 days was very poor.

Growing species: *Aspergillus repens*, *Penicillium viridicatum* Westling.

R. H. 100%: In 2 days, *Rhizopus nigricans* began to develop. In 8 days, *Aspergillus melleus*, which began to develop in 4 days, dominated over *Rhizopus* *nigricans*. Later *Penicillium variabile* and *Penicillium viridicatum* began to grow. The growth in 28 days was very good.

Dominant species: *Aspergillus melleus* Yukawa.

Subdominant species: *Penicillium variabile* Sopp, *Penicillium viridicatum* Westling.

Minor species: *Rhizopus nigricans* Ehrenberg, *Aspergillus versicolor* (Yuill.) Tirtaboschi

Kamitsure, CHAMOMILLA (J. P. VI), flower of *Matricaria chamomilla* Linné (Compositae).

R. H. 75%: No fungal growth in 28 days.

R. H. 84%: In 10 days, *Aspergillus restrictus* began to develop. The geowth in 28 days was poor.

Growing species: *Aspergillus restrictus* G. Smith.

R. H. 100%; *Asgergillus repens*, *Aspergillus ochraceus*, and *Rhizopus nigricans* began to develop in 4, 6, and 10 days, respectively. *Rhizopus nigricans* predominated over other fungi in 14 days. In 28 days, the fungal development was good.

Dominant species: *Rhizopus nigricans* Ehrenberg.

Subdominant species: *Aspergillus ochraceus* Wilhelm.

Minor species: *Aspergillus repens*, *Aspergillus luchuensis* Inui.

Very minor species: *Aspergillus oryzae* (Ahlb.) Cohn, *Aspergillus versicolor* (Yuill.) Tiraboschi.

Tôhimatsu, AURANTII PERICARPIUM PULVERATUM (J. P. VI.), pericarp of *Citrus aurantium* Linné subsp. *amara* Engler (Rutaceae).

R. H. 75%: No fungal growth in 28 days.

R. H. 84%: No fungal growth in 28 days.

R. H. 100%: In 6 days, *Aspergillus awamori* began to develop and later dominated. In 8 to 11 days *Penicillium purpurogenum* and *Penicillium implicatum* began to appear. The fungal growth in 28 days was very good.

Dominant species: *Aspergillus awamori* Nakazawa.

Subdominant species: *Penicillium purpurogenum* Stoll, *Penicillium implicatum* Biourge.

Minor species: *Aspergillus versicolor* (Vuill.) Tiraboschi.

Nihonkeihi, CINNAMOMUM LOUREIRII (J. P. VI.), cortex of *Cinnamomum loureirii* Nees (Lauraceae).

R. H. 75%: No fungal development in 28 days.

R. H. 84%: In 19 days, *Aspergillus repens* began to develop. The growth was very poor in 28 days.

Growing species: *Aspergillus repens.*

R. H. 100%: In 4 days, *Aspergillus repens* began to develop. In 20 days, *Penicillium variabile* began to grow. The growth in 28 days was good.

Dominant species: *Aspergillus repeds.*

Subdominant species: *Penicillium variabile* Sopp.

Minor species: *Aspergillus versicolor* (Vuill.) Tiraboschi.

Senkyû, CNIDIUM (N. F. J. II), subterranean stem of *Cnidium officinale* Makino (Umbelliferae).

R. H. 75%: In 12 days, *Aspergillus ruber* and *Torula sacchari* began to develop. The growth was poor in 28 days.

Growing species: *Aspergillus ruber*, *Torula sacchari* Corda.

R. H. 84%: In about 5 days, *Aspegillus mangini*, *Torula sacchari*, and *Aspergillus versicolor* began to develop. The growth in 28 days was good.

Dominant species: *Aspergillus mangini* (Mangin) Thom and Raper.

Subdominant species: *Aspergillus versicolor* (Yuill.) Tiraboschi.

Minor species: *Torula sacchari* Corda.

R. H. 100%: In one day, *Aspergillus repens* began to develop. *Rhizopus nigricans*, *Aspergillus sclerotiorum* began to develop in about 5 days and the former dominated over other fungi soon. In 10 days, *Penicillium roqueforti* began to develop under *Rhizopus nigricans.* The growth in 28 days was very good.

Dominant species: *Rhizopus nigricans* Ehrenberg, *Aspergillus sclerotiorum* Huber.

Minor species: *Aspergillus repens*, *Penicillium roqueforti* Thom, *Aspergillus niger* van Tieghem.

Saishin, Asiasari Radix, subterranean stem and root of *Asiasarum sieboldi* F. Maekawa (Aristolochiaceae).

R. H. 75%: In 18 days, *Aspergillus mangini* began to develop. The growth in 28 days was poor.

Growing species: *Aspergillus mangini* (Mangin) Thom and Raper.

R. H. 84%: In 7 days, *Aspergillus glaucus* gruop began to develop. The growth in 28 days was poor.

Growing species: *Aspergillus mangini* (Mangin) Thom and Raper, *Aspergillus* *repens*.

R. H. 100%: In 4 days, *Rhizopus nigricans*, *Aspergillus repens*, and *Aspergillus sclerotiorum* began to develop. *Rhizopus nigricans* dominated over other fungi in 7 days. The growth in 28 days was very good.

Dominant species: *Rhizopus nigricans* Ehrenberg.

Minor species: *Aspergillus repens*, *Aspergillus sclerotiorum* Huber, *Aspergillus terreus* Thom, *Aspergillus oryzae* (Ahlb.) Cohn, *Aspergillus niger* van Tieghem, *Memnoniellua echnate* (Rivolta) Galloway.

Very minor species: *Scopulariopsis brevicaulis* var. *glabrum* Thom.

Kijitsu, AURANTII IMMATRI FRUCTUS (N. F. J. II), immature fruit of *Citrus aurantium* Linné subsp. *amara* Engler, *C. aurantium* Linné subsp. *natsudaidai* Hayata, *C. aurantium* Linné subsp. *nobilis* Makino (Rutaceae) or tneir allied plants.

R. H. 75%: No fungal growth was observed in 28 days.

R. H. 84%: In 7 days, *Aspergillus ruber* began to develop. The growth in 28 days was poor.

Growing species: *Aspergillus ruber*.

R. H. 100%: In 1 day, *Aspergillus ruber* began to develop and dominated until about 7 day. Later *Aspergillus awamori* and *Aspergillui oryzae* developed rapidly. The growth in 28 days was very good.

Dominant species: *Aspergillgs awamori* Nakazawa.

Subdominant species: *Aspergillus sclerotiorum* Huber, *Aspergillus oryzae* (Ahlb.) Cohn, *Aspergillus ruber*, *Penicillium variabile* Sopp.

Tôki, LIGUSTICUM (N. F. J. II), root of *Ligusticum acutilobum* Siebold et Zuccarini (Umbelliferae).

R. H. 75%: In 28 days, no fungal development was observed.

R. H. 84%: In 12 days, *Aspergillus mangini* began to develop. The growth in 28 days was very poor.

Growing species: *Aspergillus mangini* (Mangin) Thom and Raper.

R. H. 100%: In 7 to 10 days, *Aspergillus mangini*, *Aspergillus oryzae*, and *Penicillium citrinum* began to develop. The growth in 28 days was poor.

Dominant soecies: *Aspergillus oryzae* (Ahlb.) Cohn.

Subdominant species: *Aspergillus mangini* (Mangin) Thom and Raper, *Aspergillus elegans* Gasperini, *Aspergillus niger* van Tieghem.

Minor species: *Penicillium citrinum* Thom.

Shukusha, AMOMUM XANTHIOIDES (N. F. J. II), seed of *Amomum xanthioides* Wallich (Zingiberaceae).

R. H. 75%: No fungal growth was observed in 28 days.

R. H. 84%: No fungal growth was observed in 28 days.

R. H. 100%: In 8 to 10 days, *Aspergillus ruber*, other *Aspergillus* spp., and *Rhizopus nigricans* began to develop. Until 20 days, *Aspergillus ruber* was predominant, but later *Aspergillus humicola* dominated. The growth in 28 days was poor.

Dominant species: *Aspergillus humicola* Chaudhuri and Sachar.

Minor species: *Aspergillus ruber*, *Aspergillus oryzae* (Ahlb.) Cohn, *Aspergillus niger* van Tieghem, *Aspergillus melleus* Yukawa, *Rhizopus nigricans* Ehreberg.

Kôbushi, CYPERUS (N. F. J. II), Subterranean stem of *Cyperus rotundus* Linné (Cyperaceae).

R. H. 75%: No fungal development in 28 days.

R. H. 84%: In 7 days, *Aspergillus mangini* appeared. The growth in 28 days was very poor.

Growing species: *Aspergillus mangini* (Mangini) Tnom and Raper.

R. H. 100%: Until 14 days, *Aspergillus umbrosus* which appeared in 3 days predominated. Later *Penicillium frequentans* appeared and developed rapidly. The growth in 28 days was good.

Dominant species: *Penicillium frequentans* Westling.

Minor species: *Aspergillus umbrosus* Bain. and Sart., *Aspergillus awamori* Nakazawa, *Aspergillus flavus* Link, *Aspergillus sclertiorum* Huber.

** Japanese Pharmacopeia Ed. VI.

*** National Formulary of Japan Ed. II.

Discussion and conclusion

Summarizing up the fungal spoilage of 30 kinds of crude drugs in different conditions, it is said that the most important factor to determine the spoilage type is relative humidity with which the crude drug is in equilibrium. Some of the ingredients of crude drugs are known to have the inhibitory effect upon the growth of bacteria and fungi.[1] Such crude drug as Chōji could not permit the growth of fungi at R. H. 100% in 28 days. The inhibitory effect of the ingredients may be not manifested in some cases because of the relatively low concentrations in the crude drugs. Another factor which may contribute to the protection of fungal spoilage may be sanitation that reduce the amount and kinds of fungal infection in the preparation and storage. Intrinsically, it is difficult to ask such a countermeasure in the commercial production of crude drugs.

The relative humidity which is most influential to the fungal development is expressed, in other words, as equilibrium relative humidity (e. r. h.) of the system.[2] Equilibrium relative humidity is evidently related to a factor which has a profound influence in the selection of the particular association of fungi appearing on a crude drug. The lowest values of the e. r. h. permitting the development of the spoilage fungi are listed in table 1. From this table, it is found out that *an equilibrium relative humidity of 70% gives reasonable protection against fungal development on crude drugs*, and 'alarm water content', i. e., the highest water content at which fungal growth will not occur, corresponding to this value may be calculated from water sorption isotherms.

Table 1. Relation between minimum e. r. h. and spoilage fungi

minimum e. r. h.	fungi which may appear on crude drugs	group of fungi based on their moisture requirement[3]
70% or below	no fungal development	
70%-75%	*Aspergillus glaucus*	xerophylic fungi
	Torula sacchari (*Catenularia fuliginea*)	
80%-90%	*Aspergillus* spp.	mesophylic fungi
	some spp. of *Penicillium* (mainly belonging to Biverticillata-Symmetrica)	
	Penicillium frequentans	
	Penicillium cyclopium	
over 90%	many spp. of *Penicillium*	hydrophylic fungi
	Scopulariopsis brevicaulis	
	Trichothecium roseum	
	Trichoderma viride	
	many spp. of Mucorales	

Another phenomenon observed in the fungal development on crude drugs is the 'successive appearance of fungi'. The factors influencing this phenomenon may be the gradual absorption of water by the substrate, difference of growth rate of fungi, antagonism, symbiosis, synergism, or combinations of these.

The establishment of one fungus on a substrate may influence in some ways, for example, the production of metabolic water which may disturb the local e. r. h. sufficiently to make it critical or changing the substrate nutritionally in favour of the establishment of other successing fungi. More detailed investigations will be necessary to clarify this complicated situation.

Another interesting problem is the effect of spoilage fungi, or the fungal enzymes on the ingredients of crude drugs. Further investigation will be carried on this problem in future.

The authors wish to express their appreciation to Mr. K. Tsubaki, Nagao Institute, for valuable advices and to Mr. M. Asano, Takashimadō Pharmacy, for supplying the samples.

Literature cited

1) Okazaki, K., et al. 1950-1951. Antibacterial activity of higher plants. 1-7, 19. Journ. Pharm. Soc. Jap. 70-71.

2) Mossel, D. A. A., and M. Ingram. 1955. The physiology of the microbial spoilage of food. J. appl. Bact. **18**: 232-268.

3) Semeniuk, G. 1954. Storage of ceral grains and their products. Chapter III. Microflora. Amer. Association of Cereal Chemists. St. Paul, Minnesota, U. S. A.

Errata of part 1 of this series (vol. 32 No. 7. 1957)

page	line	for	read
202	9	KCl and NaCl	NaCl and KCl
204, 205		*Torula saccharii*	*Torula sacchari* Corda

○クロミウスノキとは何か? (水島正美) Masami MIZUSHIMA: On the status of *Vaccinium hirtum* var. *atrum* Koidz.

小泉博士はウスノキに比べて花数多く，葉裏中肋に白密毛を布き，果実が黒熟するものとして，クロミウスノキと云うものを 1915 年に記載された。これは羽前東置賜（ヒガシオキクマ）郡和田村で自身採集された標本に基いている。中井博士はこれをアオバウスノキ，即ち関東地方以西の低山地産のスノキ（*Vacc. versicolor* Nak. var. *glabrum* Hara の形）と同一物と見なされ（大日本樹木誌，改訂版，1927 年），原博士はオオバスノキの変種に移され（種子植物集覧，第 1 冊，1949 年），大井博士も同様にオオバスノキの変種と見られたがウスノキもヤナギバスノキも全部同物として扱われた（日本植物誌，1953 年）。